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Polyclonal B Cell Chronic Lymphoproliferative Disease With Hairy Cell Morphology: A Case Report and Clonal Studies

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We describe a patient who has a chronic polyclonal B lymphocyte proliferation with a hairy-cell appearance. A 48-year-old Japanese woman with marked splenomegaly, systemic lymphadenopathy, and leukocytosis was referred to our hospital. Laboratory examination revealed marked polyclonal IgG hypergammaglobulinemia. Morphologic examination of the patient's peripheral blood, including May-Giemsa staining and scanning electron microscopy, showed a monotonous proliferation of hairy-appearing mature lymphocytes. An immunophenotypic study revealed an expansion of cells with mature B cell antigens positive for CD11c; however, light-chain restriction was not seen. The lack of both immunoglobulin heavy-chain and T cell receptor gene rearrangements by Southern blot analysis indicated the polyclonal nature of the proliferating B cells. This was confirmed further by a clonal analysis of the patient's lymphocytes using an X-chromosome-linked restriction fragment polymorphism within the X-linked phosphoglycerate kinase (PGK) gene. Since chronic B cell lymphoproliferation with a hairy cell appearance has not been described previously, this case might be extremely rare, and has important implications for the pathogenesis of mature B cell lymphoproliferative diseases, including hairy cell leukemia. © 1996 Wiley-Liss, Inc.

Key words: polyclonal, hairy cell, B cell

INTRODUCTION

Hairy-cell leukemia (HCL) is a relatively rare disease, and thought to be a neoplasm of B lymphocytes corresponding to an intermediate stage between pre-B cells and plasma cells [1–3]. HCL has been recognized by its characteristic morphology, histochemical features, and distinct clinical picture. It is commonly thought that HCL is a neoplasm of B cell lineage, since leukemic cells of HCL have a monoclonal surface immunoglobulin (Ig) [4] and show rearrangement of Ig genes [5]. In addition to the expression of monoclonal surface Ig and pan B cell markers, HCL shows characteristic antigen expression such as CD25, the interleukin-2 (IL-2) receptor, and CD11c, a marker associated with myelomonocytic cells [3,5,6].

We describe here a patient with a polyclonal chronic B cell proliferation with a CD11c surface phenotype. Morphologic studies, including scanning electron microscopy, showed a typical hairy appearance. Our patient shared many similarities with those with HCL reported in Japan. However, the expression of surface Ig and the analysis of Ig gene rearrangements indicated that the proliferating cells in this patient are polyclonal B cells. The polyclonality was confirmed further by analyzing the genomic DNA from the cell fraction, using conventional Southern blot analysis with a phosphoglycerate kinase (PGK) probe [7].

CASE REPORT

A 48-year-old Japanese woman was seen and admitted to Kameda General Hospital in June 1992, for evaluation

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and treatment of splenomegaly and leukocytosis. The patient had no history of cigarette smoking and was doing well until admission. Physical examination revealed massive splenomegaly, 8 cm below the left costal margin with small, palpable lymph nodes in her neck and axillae. Her leukocyte count was 23,600/μl with 92% atypical lymphocytes, 2% monocytes, and 6% polymorphonuclear leukocytes. No blasts were noted. Her hemoglobin was 9.2 g/dl, and platelet count was 98,000/μl. Coagulation studies were normal. Liver function tests such as s-GOT, s-GPT, LDH, ALP, and γ-GTP were within normal range. Serum protein was 8.9 g/dl (albumin 3.1 g/dl, globulin 5.8 g/dl). Serum electrophoresis showed a polyclonal hypergammaglobulinemia with an IgG of 4,867 μg/dl, an IgA of 16 μg/dl, and an IgM of 465 μg/dl. An M-protein band was not seen in either urine or sera by immunoelectrophoresis. Tests for antinuclear antibody and rheumatoid factors were negative. Bone marrow was aspirated easily and showed a normocellular marrow with heavy infiltration of medium-sized, atypical lymphocytes. The lymphoid cells observed in the bone marrow were identical in appearance to those seen in the peripheral blood. A bone marrow trephine biopsy did not show myelofibrosis by silver staining. Cytogenetic analysis of bone marrow cells showed a normal female karyotype. Viral titers for Epstein-Barr virus (EBV) were as follows: virus capsid antigen IgG (VCA-IgG) 1:640, VCA-IgM 1:10, and Epstein-Barr nuclear antigen (EBNA) 1:20. Typing for HLA antigens revealed the patient's phenotype to be A2, A24, B51, DR4, and DR9.

A tentative diagnosis of prolymphocytic leukemia (PLL) or HCL was considered, and cyclophosphamide (100 mg/day, p.o.) was started. This led to a slow fall in white blood cell (WBC) count to 4,000/μl and to a regression of the splenomegaly; however, the numbers of red blood cells and platelets also decreased. Cyclophosphamide was discontinued after being administered for 3 weeks, and α-INF was started without benefit. The patient remained clinically well without treatment for 36 months after diagnosis, although her WBC count increased to 10,000/μl with 78% atypical lymphocytes.

MATERIALS AND METHODS

Morphologic Features and Ultrastructural Study

Morphologic studies were performed using standard May-Giemsa staining, and acid phosphatase staining with tartrate inhibition. Scanning electron microscopy was performed by routine methods [8].

Immunophenotype Determinations

Surface marker analysis was performed by flow cytometry, using a wide panel of monoclonal antibodies. The T cell-related monoclonal antibodies included: CD2 (Leu5), CD3 (Leu4), CD4 (Leu3), CD5 (Leu1), CD7 (Leu9), and CD8 (Leu2). The B cell-related monoclonal antibodies included: CD10 (CALLA), CD19 (Leu12), CD20 (Leu16), CD22 (Leu14), and PCA-1. Other monoclonal antibodies included: CD11c (LeuM5), CD13 (My7), CD14 (Mo2), CD33 (My9), CD34 (My10), HLA-DR, and CD25 (IL-2R). Fluorescence-labeled polyclonal mouse anti-human Igs for Igκ and Igλ were also used for analysis.

Mononuclear cells were isolated by Lymphoprep (Nyegaard and Co., Oslo, Norway) gradient centrifugation. The cells were treated with 5% heat-aggregated human IgG to block nonspecific binding to the Fc receptor. Indirect immunfluorescence was used for staining with monoclonal antibodies, and the cells were separated into subpopulations based on forward and light scatter characteristics. Two-color analysis was performed by use of fluorescence (FITC)-conjugated and phycoerythrin (PE)-conjugated monoclonal antibodies. Five thousand–10,000 cells were analyzed for each monoclonal antibody used, and the actual percentage of cells positive for each monoclonal antibody was calculated by subtraction of the background and nonspecific labeling with an Ig-type-specific mouse monoclonal antibody.

Gene Rearrangement Study

High molecular weight DNA was extracted from the mononuclear cells and digested to completion with *Eco*RI, *Hind*III, or *Bam*HI restriction endonucleases. The DNA was size-fractionated by electrophoresis through a 0.8% agarose gel and transferred to nylon filters. Southern blot analysis was performed as previously reported [9]. The DNA probes, JH for the joining (J) region gene of the Ig heavy chain, and Cβ1 for the constant (C) region gene of the T cell receptor (TCR) β, were used for this analysis. To test for the presence of the EBV genome, a probe which consisted of a 5.2-kb *Bam*HI-*Eco*RI fragment containing the tandem terminal repeated sequences of the EBV genome [10] was used.

Clonal Analysis of X-Linked Gene

Purified granulocytes and mononuclear cells were used as two separate sources of DNA. High molecular weight DNA was extracted by standard procedures. The experimental protocols used for the clonal analysis of X-linked genes have been described elsewhere [7,11]. Briefly, for PGK gene analysis, 30 μg of DNA was digested first with *Eco*RI, *Bgl*I, and *Bgl*II separately. These samples were divided into two aliquots, one of which was further digested with *Hpa*II. These were subjected to electrophoresis in a 1.5% agarose gel for 6 hr. After electrophoresis, the DNA was transferred and fixed onto a Hypond-N+ (Amersham, Aylesbury, UK) membrane. The membrane was hybridized with a ^{32}P-labeled probe of the PGK gene (kindly supplied by Dr. J. Singer-Sam, Beckman Research Institute, City of Hope, CA), washed, and then autoradio-

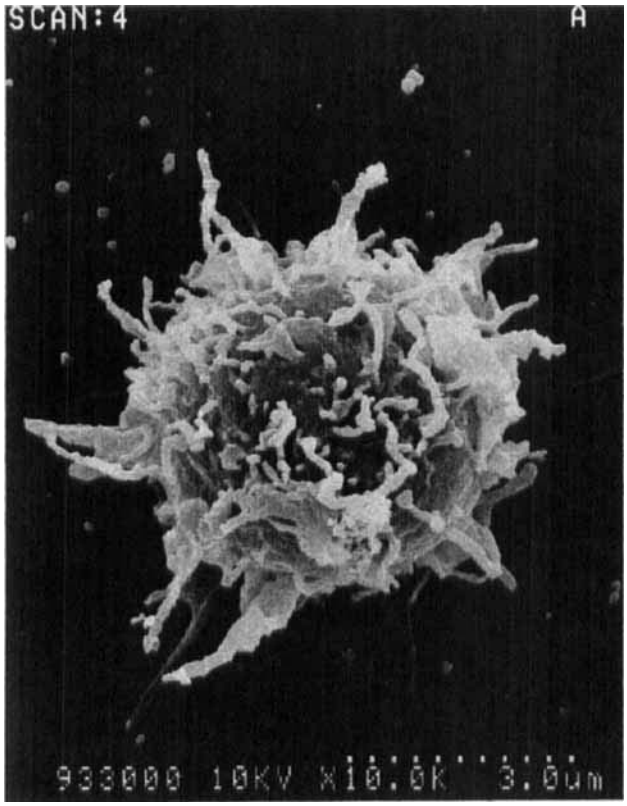


Fig. 1. Scanning electron microscopic observation of a peripheral blood mononuclear cell (×6,800), separated by density gradient centrifugation, with prominent ruffles and microvilli.

graphed. The PGK probe used in this study was a 0.8-kb *Bam*HI/*Eco*RI fragment derived from plasmid pSPT19.1.

RESULTS

Morphologic Features

Examination of the peripheral blood showed marked lymphocytosis with a homogeneous appearance. The cells were intermediate in size with abundant, faintly basophilic cytoplasm that stained homogeneously. Cytoplasmic granules were not evident. The nuclei were round to oval, and the chromatin was coarsely clumped. The nucleoli were inconspicuous. The cytoplasmic border did not show the typical villous projections under the light microscope in the well-stretched areas of the smear, although some cells with scalloped borders were seen occasionally. Scanning electron microscopic examination of the peripheral blood showed that the membrane was adorned with numerous broad-based ruffles and ridge-like processes (Fig. 1) typically seen in HCL. Tartrate-resistant acid phosphatase (TRAP) activity was weakly present.

Immunophenotype

The results of the flow cytometric analysis are summarized in Table I. Most of the mononuclear cells were positive for B cell-associated antigens such as CD19, CD20, CD21, and HLA-DR, although negative for PCA-1. The intensity of the surface Ig was moderate, and the light-chain κ/λ ratio did not suggest a clonal B cell expansion. The CD5 and CD25 antigens, which are usually positive in chronic lymphocytic leukemia (CLL) and HCL, respectively, were negative. With the exception of the CD11c antigen which is specific for HCL, other markers of T cells, myelomonocytes, and NK cell-associated antigens were negative. The CD11c antigen was positive in most of the CD20-positive cells, as confirmed by two-color flow cytometric analysis.

Gene Rearrangement Analysis

Rearranged TCR bands were not seen in any of the *Eco*RI, *Bam*HI, or *Hind*III digests of DNA from the patient's peripheral blood mononuclear cells. In an Ig heavy-chain gene rearrangement study, a faint 23.1-kb *Eco*RI band was observed. However, no rearranged band was seen using other restriction endonucleases such as *Hind*III, and *Bam*HI plus *Hind*III. Because of the lack of any rearranged band in the *Bam*HI plus *Hind*III digestions, the faint 23.1-kb band observed with *Eco*RI digestion was considered nonspecific (Fig. 2). Southern blot analysis for EBV DNA did not detect any rearranged band, and indicated the absence of clonal EBV genomes.

Clonal Analysis of X-Linked Gene

Figure 3 shows the results of the Southern blot analysis for X-inactivation of the PGK gene. The patient's granulocytes and mononuclear cells after *Hpa*II digestion both exhibited a two-band pattern identical to that before the digestion, indicating polyclonality of both cell fractions.

DISCUSSION

Clonal B cell lymphoproliferative diseases are a group of disorders with diverse clinical and pathologic manifestations. Of these, HCL is a well-characterized clinical entity in its clinical and immunological aspects, and is thought to be a B cell neoplasm. HCL is an extremely rare disease in Japan, and in previous reports Japanese patients have shown considerable differences in their clinical manifestations compared with those of patients in Western countries [12,13]. Recently, Machii et al. [14] reported a predominance of a distinct subtype of HCL in Japan. These patients have a relatively high white cell count, a weakly positive TRAP, an easily obtained bone marrow aspirate, and cells positive for CD11c and negative for CD25. They proposed to term this disorder an HCL Japanese variant. Our case shared many similarities

TABLE I. Immunophenotypic Analysis of Peripheral Blood Mononuclear Cells*

T cell lineage		B cell lineage		Myeloid lineage		Others	
CD2	8	CD10	0	CD13	2	CD25	0
CD3	2	CD19	94	CD11c	70	HLA-DR	100
CD4	4	CD20	97	CD14	3		
CD5	4	CD21	31	CD33	2		
CD8	6	PCA-1	0				
		slgκ	30				
		slgλ	21				

*Surface antigen expression was determined by direct immunofluorescence, using a FACScan flow cytometer. Results are shown as percentages of positive cells.

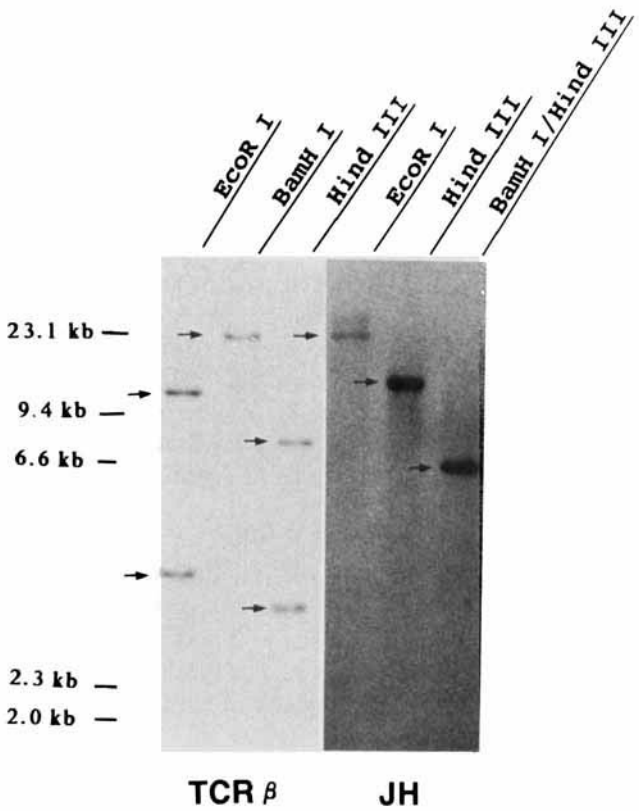
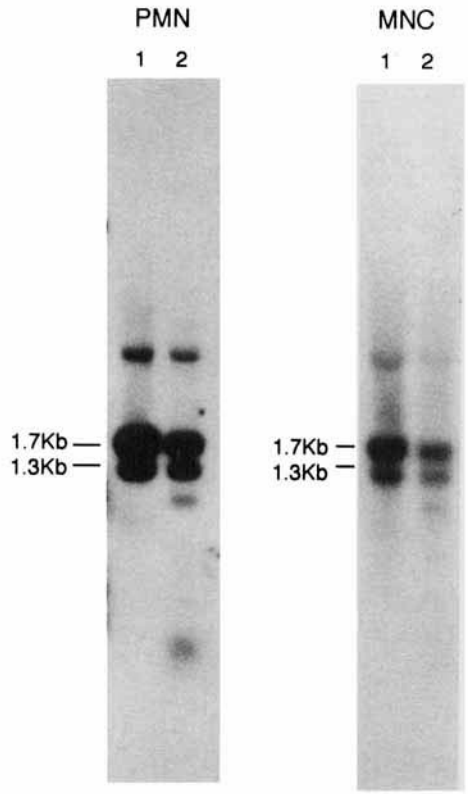


Fig. 2. Southern blot analysis of TCR gene and Ig heavy-chain gene rearrangement. Genomic DNA extracted from peripheral blood mononuclear cells was digested with *Eco*RI, *Bam*HI, and *Hind*III restriction enzymes and hybridized with probes for TCRβ and JH. Arrows indicate germ line bands with each restriction enzyme.



lane1, Bgl*I* polymorphism
lane2, Hpa*II* digestion

Fig. 3. Clonal analysis using a PGK probe, showing polyclonal appearance of DNA from peripheral blood granulocytes and mononuclear cells. Lane 1: *Bgl*I RFLP. Lane 2: DNA digested with both *Bgl*I and *Hpa*II. PMN, polymorphonuclear cells; MNC, mononuclear cells.

with these HCL Japanese variant cases reported by Machii et al. [14] in terms of their clinical, morphologic, and immunologic aspects. However, our patient showed a marked polyclonal hypergammaglobulinemia that was uncommon in the HCL Japanese variant. In addition, the lack of Ig light-chain restriction and the lack of Ig heavy-chain gene rearrangement indicate that the proliferating, hairy-appearing cells in our patient were polyclonal in origin. This polyclonality was supported further by an analysis of restriction fragment length polymorphisms and X-inactivation at the PGK gene. Thus, our patient should not be diagnosed as having a leukemia, but should be considered to have a polyclonal chronic B lymphoproliferative disease with hairy-cell morphology.

Expression of CD11c is thought to be one of the characteristic immunologic markers of HCL among the B cell

neoplasms. However, occasional cases of CLL have been reported to express CD11c. Hanson et al. [15] have reported a distinct CD11c-positive, chronic B cell lymphoproliferative disorder with features of CLL and HCL. Our patient shared many clinical findings with the patients of Hanson et al. [15], including an indolent clinical course, and easily aspirated bone marrow. However, all their patients had a light-chain restriction in surface Ig suggesting the monoclonal expansion of B cell in their blood.

Persistent polyclonal B lymphocytosis with a polyclonal increase in serum IgM has been described in female cigarette smokers [16–18]. Blood smears revealed the presence of atypical lymphocytes with characteristic binucleated cells. These patients were reported to express HLA-DR7 antigen. Our patient had no history of cigarette smoking and did not express HLA-DR7 antigen in her peripheral blood lymphocytes. In addition to these morphological differences, the CD11c antigen was not expressed in abnormal lymphocytes in these cases. These aspects of this case are clearly different from previously reported cases of persistent polyclonal B lymphocytosis. Polyclonal B cell proliferation is also seen in patients with an organ transplant [19] or with immunodeficiency [20], and usually is associated with Epstein-Barr virus (EBV) infection [10,19,20]. Despite the neoplastic nature of this B cell lymphoproliferation and the presence of a clonal EBV genome, the clonality of the proliferating cells is not always clear. Because our patient did not have EBV DNA in her peripheral blood by Southern blot analysis, it is unlikely that an active EBV infection played a role in the proliferation of the hairy B lymphocytes in our patient. However, the involvement of other viruses, such as a retrovirus, might have an etiologic role in the development of the proliferation of hairy-appearing B cells. Indeed, Kalyanaraman et al. [21] and Rosenblatt et al. [22] have reported the isolation of human T cell lymphotrophic virus type II (HTLV-II) from patients with atypical hairy-cell leukemia with a T cell phenotype.

In conclusion, we report here on a patient with a hairy cell-like B cell proliferation that has not been described previously. The patient shares many similarities with the HCL Japanese variants; however, her laboratory findings, which include polyclonal hypergammaglobulinemia, lack of light-chain restriction in surface Ig expression, and absence of Ig gene rearrangement, suggest a polyclonal nature of the proliferating B cells in our patient. Clonal studies using restriction fragment length polymorphism (RFLP) of the X-linked PGK gene also support the polyclonality of these B cells.

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REFERENCES

1. Melo JV, Catovsky D, Galton DAG: The relationship between chronic lymphocytic leukemia and prolymphocytic leukemia. I. Clinical and laboratory features of 300 patients and characterization of an intermediate group. Br J Haematol 63:377, 1986.
2. Gale RP, Foon KA: Chronic lymphocytic leukemia. Recent advances in biology and treatment. Ann Intern Med 103:101, 1985.
3. Anderson KC, Boyd AW, Fisher DD, Leslie D, Schlossman SF, Nadler LM: Hairy cell leukemia: A tumor of pre-plasma cells. Blood 65:620, 1985.
4. Robbins BA, Ellison DJ, Spinosa JC, Lukes RJ, Poppema S, Saven A, Piro LD: Diagnostic application of two-color flow cytometry in 161 cases of hairy cell leukemia. Blood 82:1277, 1993.
5. Korsmeyer SJ, Greene W, Cossman WC, Hsu S-M, Jansen JP, Neckers LM, Marshall SL, Bakhshi A, Depper JM, Leonard WJ, Jaffe ES, Waldmann TA: Rearrangement and expression of immunoglobulin genes and expression of Tac antigen in hairy cell leukemia. Proc Natl Acad Sci USA 80:4522, 1983.
6. Schwarting R, Stein H, Wang CY: The monoclonal antibodies αS-HCL1 (αLeu14) and αS-HCL3 (αLeu-M5) allow the diagnosis of hairy cell leukemia. Blood 65:974, 1985.
7. Vogelstein B, Fearon ER, Hamilton SR, Preisinger AC, Willard HF, Michelson AM, Riggs AD, Orkin SH: Clonal analysis using recombinant DNA probe from the X chromosome. Cancer Res 47:4806, 1987.
8. Inoue R, Taniguchi N, Konishi I, Machii T, Kitani T: A SEM study on the surface architecture of leukemic cell in lymphoproliferative disorders. J Clin Electron Microsc 16:5, 1983.
9. Cleary ML, Chao J, Wranke R, Sklar J: Immunoglobulin gene rearrangement as a diagnostic criterion of B cell lymphoma. Proc Natl Acad Sci USA 81:593, 1984.
10. Kaplan MA, Ferry JA, Harris NL, Jacobson JO: Clonal analysis of posttransplant lymphoproliferative disorders, using both episomal Epstein-Barr virus and immunoglobulin genes as markers. Am J Clin Pathol 101:590, 1994.
11. Jowitt SN, Liu Yin JA, Sauders MJ, Lugas GS: Clonal remissions in acute myeloid leukemia are commonly associated with features of trilineage myelodysplasia during remission. Br J Haematol 85:698, 1993.
12. Kitani T, Machii T, Inoue R, Kanakura Y: Hairy cell leukemia. A report of ten cases in Japan and characterization of anti-hairy cell sera. Jpn J Clin Oncol 13:497, 1983.
13. Katayama I, Hirashima K, Maruyama K, Hoshino S, Abe T, Furusawa S, Iguchi Y: Hairy cell leukemia in Japanese patients: A study with monoclonal antibodies. Leukemia 1:301, 1983.
14. Machii T, Tokumine Y, Inoue R, Kitani T: Predominance of a distinct subtype of hairy cell leukemia in Japan. Leukemia 7:181, 1993.
15. Hanson CA, Gribbin TE, Schnitzer B, Schlegelmilch JA, Mitchell BS, Stoolman LM: CD11c (Leu-M5) expression characterizes a B-cell chronic lymphoproliferative disorder with features of both chronic lymphocytic leukemia and hairy cell leukemia. Blood 76:2360, 1990.
16. Gordon DS, Jones BM, Browning SW, Spira TJ, Lawrence DN: Persistent polyclonal lymphocytosis of B lymphocytes. N Engl J Med 307:232, 1982.
17. Carstairs KC, Francombe WH, Scott JG, Gelfand EW: Persistent polyclonal lymphocytosis of B lymphocytes, induced by cigarette smoking? Lancet ii:1094, 1985.
18. Troussard X, Valensi F, Debert C, Maynadie M, Schillinger F, Bonnet P, Macintyre EA, Flandrin GF: Persistent polyclonal lymphocytosis with binucleated B lymphocytes: A genetic predisposition. Br J Haematol 88:275, 1994.
19. Hanto DW, Birkenbach M, Frizzera G, Gajl-Peczalska KJ, Simmons RL, Schubach WH: Confirmation of the heterogeneity of posttransplant

Epstein-Barr virus-associated B cell proliferations by immunoglobulin gene rearrangement analysis. Transplantation 47:458, 1989.

20. Suber M, Neri A, Inghirami G, Knowles D, Dalla-Favera R: Frequent c-myc oncogene activation and infrequent presence of Epstein-Barr virus genome in AIDS-associated lymphoma. Blood 72:667, 1988.
21. Kalyanaraman VS, Sarngadharan MG, Robert-Guroff M, Miyoshi I, Blayney D, Golde D, Gallo RC: A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T cell variant hairy cell leukemia. Science 218:571, 1982.
22. Rosenblatt JD, Golde DW, Wachsman W, Giorgi JV, Jacobs A, Schmidt GM, Quan S, Gasson JC, Chen ISY: A second isolation of HTLV-II associated with atypical hairy cell leukemia. N Engl J Med 315:372, 1986.